

EDITORIAL



Dendritic cells as natural latency reversing agent: A wake-up call for HIV-1

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Combination antiretroviral therapy blocks HIV-1 replication, allowing the immune system to clear the virus producing cells. This reduces new HIV-1 particle production to undetectable levels. Alas, latent HIV-1 infections frequently occur in activated and memory T cells, macrophages, dendritic cells (DCs) and other susceptible cell types.^{1,2} These latently infected cells form latent reservoirs because they are not removed via cytotoxic T cell killing due to the lack of viral antigen presentation. The reservoir in patients on therapy mostly consists of long-lived resting memory T cells^{3,4} as activated T cells have a very short half-life. Monocytes and DCs have long half-lives, but their presence in the latent reservoir is strongly reduced after 6 months of therapy.^{5,6} Long-lived latently infected cells that harbor replication competent virus can be triggered to start virus production as evidenced by a rapid viral rebound when combined antiretroviral therapy is stopped.^{5,7} Ren *et al.* have shed light on how this triggering might occur.⁸ They demonstrated that dendritic cells, when matured upon T cell contact or via stimulation with bacterial compounds, are capable of purging latently infected T cells via secretion of TNF α . These results highlight that bacterial co-infections can modulate HIV-1 reservoirs via dendritic cell maturation.

It is unknown why some cells are latently infected while others allow productive virus replication. Studying the influence of drugs to purge latency by activating signal transduction cascades can provide insight in the molecular mechanisms of latency establishment and the possibility of therapeutic purging. Such drugs are known as latency reversing agents (LRAs) and were reviewed in Spina *et al.*⁹ Prostratin or bryostatin activate the protein kinase C (PKC) pathway and reverse latency by increasing active NF- κ B levels.¹⁰ TNF α induces the MEK-ERK and I κ K pathways and activate viral transcription by elevating the level of active NF- κ B and c-Fos/c-Jun.¹¹ The

HDAC inhibitors, romidepsin and vorinostat can remodel the chromatin structure.^{12,13} They act as LRA by increasing the binding accessibility of transcription factors to the HIV-1 long terminal repeat (LTR) promoter region, thus boosting viral transcription. However, the strongest LRAs are T cell receptor (TCR) agonists such as phytohaemagglutinin (PHA) and CD3/CD28 antibodies. These agonists drive clonal T cell proliferation and activate multiple signaling cascades such as the MEK-ERK, PKC, I κ K, PI3K-Akt and other pathways.¹⁴

Tests of various LRAs on resting memory cells from patients indicated striking differences in the efficiency of latency purging.^{9,13,15,16} Per million resting T cells, maximally 3 cells could be converted to produce virus by repeated TCR stimulation with PHA or CD3/CD28,^{17,18} whereas other LRAs failed with the exception of PMA/ionomycin.¹³ The estimated number of cells that contain infectious replication competent HIV-1, however, is thought to be 10-fold higher.¹⁹ Thus latency reversal with drug-based LRAs is quite inefficient.

It is not known whether the rapid viral rebound is caused by reversal of latency or by ongoing virus replication in tissues with a low drug penetration. There is evidence that virus evolution still occurs during therapy, in particular with some drug cocktails or therapy non-adherence.²⁰ Other reports show ongoing virus replication without viral evolution as the HIV-1 progeny is related to the virus present at earlier time points.^{21,22} The latter data imply that latent HIV-1 can be activated spontaneously via a natural mechanism, such as cell-cell contact or via production of latency regulating cytokines during inflammation.

A few groups have studied reversal of HIV-1 latency mediated by cell-cell interactions.^{23–25} Especially immature dendritic cells (DCs) were capable of undoing latency in TCR-activated latently infected primary T cells

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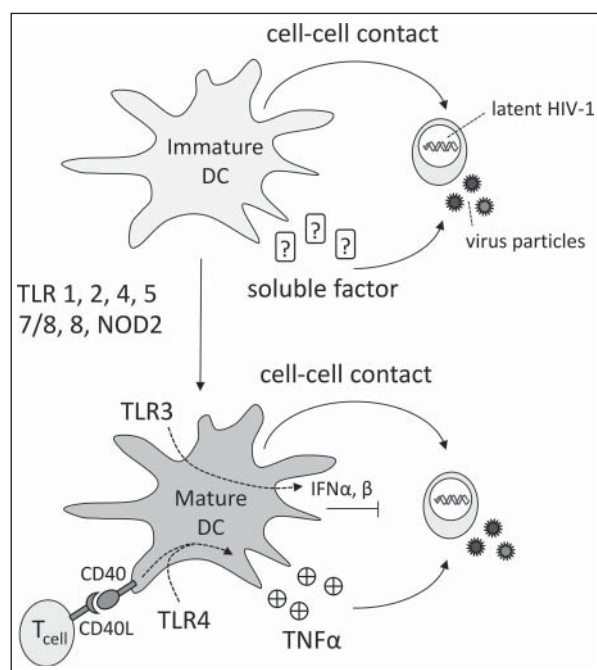


Figure 1. DC-mediated purging of latency. Immature DCs are able to revert HIV-1 latency in primary T cells upon cell-cell contact or via secretion of an unknown soluble natural LRA. Purging latent HIV-1 can also be achieved with mature DCs stimulated with TLR 1,2,4,5,7/8,8 or NOD2. TLR3 stimulated DCs probably can revert latency, but additional production of IFN α and β negate latency reversion capacities.²³ DCs can revert latency via secretion of TNF α . This can be achieved by stimulating DCs with TLR4 or upon contact with T cells in a CD40/CD40L ligand dependent manner.⁸

upon cell-cell contact and via secretion of an unknown soluble factor (Fig. 1). Macrophages were also capable of overcoming HIV-1 latency, but are not as potent as DCs, whereas B cells, T cells or monocytes did not purge latent HIV-1.²³ Studying the anti-latency properties of DCs derived from various tissues showed differential activities: genital tract DCs like dermal DCs and plasmacytoid DCs could not revert latency, whereas representative gut DCs purged latent HIV-1 efficiently.²³ Maturation of DCs with different Toll-like receptor (TLR 1, 2, 4, 5, 7/8, 8) agonists or NOD2 enabled efficient reversal of latency, with the exception of TLR3 matured DCs treated with double stranded (poly I:C) RNA. TLR3-matured DCs produce high amounts of interferons α and β , which negate the purging effect.²³ Thus latent HIV-1 can be purged naturally by DCs (Fig. 1), depending on the type of tissue and DCs present therein.

It may not be unexpected that DCs undo HIV-1 latency in T cells as the antigen presentation and recognition processes can induce T cell receptor activation and clonal expansion. Thus DCs should have similar purging properties as the most powerful LRAs, CD3/CD28 or PHA combined with HDAC inhibitors. Since

the HIV-1 reservoir in treated patients primarily consists of memory T cells recognizing highly specific antigens the chance that DCs present those antigens is slim.²⁶ Moreover, HIV-1 specific memory T cells cannot be stimulated by DCs when therapy is initiated and antigens become unavailable.

Reversion of latent HIV-1 by DCs, however, does not only work via antigen presentation pathways. Latently infected T cells can initiate virus production upon DC stimulation without antigen presentation.²⁴ In addition, DCs can secrete soluble components that have LRA activity. Now, Ren *et al.* identified TNF α as such a natural LRA secreted by DCs⁸ (Fig. 1). They show that immature DCs increase CD40 expression and mature upon contact with T cells. The matured DC subsequently releases TNF α that can initiate virus production in the latently infected cell line. Maturation of DCs could also be induced with lipopolysaccharide or *Mycobacterium bovis*, and DC-supernatant could purge resting memory T cells from infected patients. This important finding confirms that cells belonging to the patient reservoir can be purged by immature DCs via cell-cell contact or upon their maturation with bacterial products, which might be regulated via release of TNF α .^{8,23-25} Further evaluation of TNF α as LRA on primary T cells is of utmost importance. Surprisingly, in the experimental setup used by Ren *et al.*, cell-cell contact between matured DCs and T cells did not lead to reversion of HIV-1 latency, but a diminished expression of CD40 on the DC was noticed. Possibly reduced CD40 binding to its CD40 ligand partner on the T cell decreases activation of signaling cascades or TNF α release below the purging threshold. Alternatively, matured DCs can release interferons that block reversion of latent HIV-1²³ or upregulate co-inhibitory molecules such as PDL1/PDL2 that might block reversal of latency.²⁷

Drug-based LRAs provided insights on how to purge latent HIV-1, but the use of these drugs to reduce the reservoir in patients is either blocked by toxicity or the drugs are not sufficiently effective to allow one to stop treatment.^{28,29} Not much is known about natural mechanisms that can influence activation of latently infected cells and their contribution to infection reestablishment. As proven by Ren *et al.* and others, DCs can purge latency, either via cell-cell interactions or via release of natural LRAs.^{8,23-25} Understanding what natural signaling pathway is required to purge latently infected cells in patients might help to develop drugs that mimic a natural purging mechanism to accelerate reservoir decay towards thresholds allowing therapy termination. Alternatively, drugs could be developed that prevent DC-mediated purging. In the end, such drugs might forever prevent HIV-1 from awakening.

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